FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **SALK1470-2**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/155252**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US96/05465	**18 April 1996**	**25 April 1995**

TITLE OF INVENTION

SELECTIVE MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF

APPLICANT(S) FOR DO/EO/US

RONALD M. EVANS et. a.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

    Petition to Revive
    Post Card

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/US96/05465	SALK1470-2

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Search Report has been prepared by the EPO or JPO	**$930.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$720.00**
☐	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$790.00**
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO	**$1,070.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$98.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$1,070.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	23 - 20 =	3	x $22.00	**$66.00**	
Independent claims	4 - 3 =	1	x $82.00	**$82.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS			=	**$1,348.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).**			☒	**$674.00**	
SUBTOTAL			=	**$674.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE			=	**$674.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☒	**$120.00**	
TOTAL FEES ENCLOSED			=	**$794.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **07-1895** in the amount of $1,454.00 to cover the above fees.
A duplicate copy of this sheet is enclosed. (above amount includes $660.00 petition fee)

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **07-1895** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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31,192
REGISTRATION NUMBER

9/21/98
DATE

Selective Modulators of Peroxisome Proliferator Activated Receptor-gamma, and Methods for the Use Thereof

FIELD OF THE INVENTION

The present invention relates to methods for the modulation of nuclear receptor mediated processes. In a particular aspect, the present invention relates to the use of a specific class of compounds for the modulation of processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). In another aspect, the present invention relates to methods of testing compounds for their ability to regulate transcription-activating effects of PPAR-γ.

BACKGROUND OF THE INVENTION

Peroxisome proliferators are a structurally diverse group of compounds which, when administered to rodents, elicit dramatic increases in the size and number of hepatic and renal peroxisomes, as well as concomitant increases in the capacity of peroxisomes to metabolize fatty acids via increased expression of the enzymes required for the ß-oxidation cycle (Lazarow and Fujiki, *Ann. Rev. Cell Biol.* 1:489-530 (1985); Vamecq and Draye, *Essays Biochem.* 24:1115-225 (1989); and Nelali et al., *Cancer Res.* 48:5316-5324 (1988)). Chemicals included in this group are the fibrate class of hypolipidermic drugs, herbicides, and phthalate plasticizers (Reddy and Lalwani, *Crit. Rev. Toxicol.* 12:1-58 (1983)). Peroxisome proliferation can also be elicited by dietary or physiological factors such as a high-fat diet and cold acclimatization.

Insight into the mechanism whereby peroxisome proliferators exert their pleiotropic effects was provided by the identification of a member of the nuclear hormone receptor superfamily activated by these chemicals (Isseman and Green, *Nature* 347-645-650 (1990)). This receptor, termed peroxisome proliferator activated receptor alpha (PPARα), was subsequently shown to be activated by a variety of medium and long-chain fatty acids and to stimulate expression of the genes encoding rat acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal ß-oxidation), as well as rabbit cytochrome P450 4A6, a fatty acid ω-hydroxylase (Gottlicher et al., *Proc. Natl. Acad. Sci. USA* 89:4653-4657 (1992); Tugwood et al., *EMBO J.* 11:433-439 (1992); Bardot et al., *Biochem. Biophys. Res. Comm.* 192:37-45 (1993); Muerhoff et al., *J. Biol. Chem.* 267:19051-19053 (1992); and Marcus et al., *Proc. Natl. Acad. Sci. USA* 90(12):5723-5727 (1993).

The above-noted references suggest a physiological role for PPARα in the regulation of lipid metabolism. PPARα activates transcription by binding to DNA sequence elements, termed peroxisome proliferator response elements (PPRE), as a heterodimer with the retinoid X receptor. The retinoid X receptor is activated by 9-*cis* retinoic acid (see Kliewer et al., *Nature* 358:771-774 (1992), Gearing et al., *Proc. Natl. Acad. Sci. USA* 90:1440-1444 (1993), Keller et al., *Proc. Natl. Acad. Sci. USA* 90:2160-2164 (1993), Heyman et al., *Cell* 68:397-406 (1992), and Levin et al., *Nature* 355:359-361 (1992)). Since the PPARα-RXR complex can be activated by peroxisome proliferators and/or 9-*cis* retinoic acid, the retinoid and fatty acid signaling pathways are seen to converge in modulating lipid metabolism.

Since the discovery of PPARα, additional isoforms of PPAR have been identified, e.g., PPARβ, PPARγ and PPARδ, which are spatially differentially expressed. Because there are several isoforms of PPAR, it would be desirable to identify compounds which are capable of selectively interacting with only one of the PPAR isoforms. Such compounds would find a wide variety of uses, such as, for example, in the prevention of obesity, for the treatment of diabetes, and the like.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified a class of compounds which are capable of selectively modulating processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). The identification of such compounds makes possible the selective intervention in PPAR-γ mediated pathways, without exerting inadvertent effects on pathways mediated by other PPAR isoforms.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the activation of a GAL4-PPARγ fusion protein by a variety of prostaglandin or prostaglandin-like compounds. In the figure, black bars represent 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-d PGJ2), the dark, striped bars represent prostaglandin-J_2 (PGJ2), the darkly shaded bars represent 9α,11β-prostaglandin-F_2 (9a,11bPGF2), the light, closely (diagonally) striped bars represent prostaglandin-I_2 (PGI2), the open bars represent prostaglandin-A_2 (PGA2), the dark bars with light dots represent prostaglandin-B_2 (PGB2), the horizontally hatched bars represent prostaglandin-D_2 (PGD2), the light bars with dark dots represent prostaglandin-E_2 (PGE2), the light, sparsely (diagonally) hatched bars represent prostaglandin-$F_{2\alpha}$ (PGF2a), and the

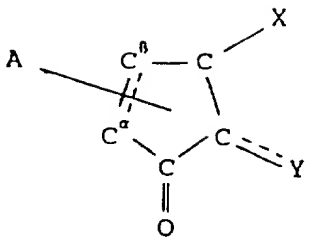
light bars with sparsely spaced dots represent bicycloprostaglandin-E_1 (BicycloE1).

Figure 2 illustrates the dose response for activation of a GAL4-PPARγ fusion protein by a variety of prostaglandin or prostaglandin-like compounds. In the figure, open circles represent prostaglandin-D_2 (PGD2), darkened circles represent prostaglandin-J_2 (PGJ2), open squares represent Δ^{12}-prostaglandin-J_2 (Δ12-PGJ2), and darkened squares represent 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-deoxy-Δ12,14-PGJ2).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for modulating process(es) mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising conducting said process(es) in the presence of at least one PPAR-γ-selective prostaglandin or prostaglandin-like compound or precursor thereof.

PPAR-γ-selective prostaglandins or prostaglandin-like compounds contemplated for use in the practice of the present invention include members of the prostaglandin-J_2 family of compounds (e.g., prostaglandin-J_2, Δ^{12}-prostaglandin-J_2 or 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2), members of the prostaglandin-D_2 family of compounds (e.g., prostaglandin-D_2), or precursors thereof, as well as compounds having the structure **I**:



(I)

wherein:

A is selected from hydrogen or a leaving group at the α- or β- position of the ring, or A is absent when there is a double bond between C^{α} and C^{β} of the ring;

X is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl group having in the range of 2 up to 15 carbon atoms; and

Y is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl group having in the range of 2 up to 15 carbon atoms.

As employed herein, the term "leaving group" refers to functional groups which can readily be removed from the precursor compound, for example, by nucleophilic displacement, under E_2 elimination conditions, and the like. Examples include hydroxy groups, alkoxy groups, tosylates, brosylates, halogens, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen,

trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, and the like.

As employed herein, "cycloalkyl" refers to cyclic ring-containing groups containing in the range of about 3 up to 8 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkylaryl" refers to alkyl-substituted aryl groups and "substituted alkylaryl" refers to alkylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkyl" refers to aryl-substituted alkyl groups and "substituted arylalkyl" refers to arylalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkynyl" refers to aryl-substituted alkynyl groups and "substituted arylalkynyl" refers to arylalkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aroyl" refers to aryl-carbonyl species such as benzoyl and "substituted aroyl" refers to aroyl groups further bearing one or more substituents as set forth above.

As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocyclic" refers to heterocyclic groups further bearing one or more substituents as set forth above.

As employed herein, "acyl" refers to alkyl-carbonyl species.

As employed herein, "halogen" or "halo" refers to fluoro substituents, chloro substituents, bromo substituents or iodo substituents.

In a presently preferred aspect of the present invention, "X" of Formula I is selected from:

$-(CRR)_m-Z$,

$-(CRR)_{m'}-C(R)=C(R)-(CRR)_{m'}-Z$, or

$-(CRR)_{m''}-C\equiv C-(CRR)_{m''}-Z$, wherein:

each R is independently selected from H, lower alkyl, substituted lower alkyl,

hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

m falls in the range of 1 up to 15,

each m' falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkenyl moiety does not exceed 15 carbon atoms,

each m" falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkynyl moiety does not exceed 15 carbon atoms, and

Z is a polar, heteroatom-containing substituent.

Those of skill in the art can readily identify numerous groups which satisfy the requirement that Z be a polar, heteroatom-containing (i.e., O, N, S, or the like) substituent. Thus, Z can be selected from cyano, nitro, amino, carbamate, or a substituent having the structure:

$-CH_2OR'$, wherein R' is selected from H, alkyl, alkenyl, alkynyl, acyl, aryl, or the like;

-C(O)R", wherein R" is selected from H, alkyl, substituted alkyl, alkoxy, alkylamino, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aryloxy, arylamino, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, heterocyclic, substituted heterocyclic or trifluoromethyl,

$-CO_2R'''$, wherein R''' is selected from H, alkyl, alkenyl, alkynyl, or the like;

$-SR'$, $-S(O)R'$, $-S(O)_2R'$ or $-S(O)_2NHR'$, wherein each R' is as defined above,

and the like.

Especially preferred compounds employed in the practice of the present invention are those wherein "X" of Formula I is

$-CRR-C(R)=C(R)-(CRR)_m-Z$, wherein:

each R is independently selected from H, lower alkyl, substituted lower alkyl, hydroxy, alkoxy (of a lower alkyl group), halogen, trifluoromethyl, amino, carboxyl or sulfonyl,

m falls in the range of 1 up to 6, and

Z is selected from $-CH_2OH$, $-CH_2OAc$, $-CO_2H$, $-CO_2Me$ or $-CO_2Et$.

In another preferred aspect of the present invention, "Y" of Formula I is selected from:

$=C(R)-[C(R)=C(R)]_n-(CRR)_{n'}-Z'$ (**II**),

$=C(R)-[C\equiv C]_{n''}-(CRR)_{n'}-Z'$ (**IIA**),

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ (**III**),

$-[C(R)=C(R)]_n-(CRR)_{n'}-Z'$ (**IV**), or

$-[C\equiv C]_n-(CRR)_{n'}-Z'$ (**IVA**),

wherein

each R is independently as defined above,

each R' is independently selected from H, lower alkyl, substituted lower alkyl or a leaving group,

Z' is selected from H, lower alkyl or substituted lower alkyl,

n falls in the range of 0 up to 4,

n' falls in the range of 2 up to 12, and

n" falls in the range of 1 up to 3.

Especially preferred compounds contemplated for use in the practice of the present invention include those wherein "Y" of Formula **I** is selected from:

$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ **(II)**,

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ **(III)**, or

$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ **(IV)**, wherein

each R is independently as defined above,

each R' is independently as defined above,

Z' is selected from H, lower alkyl or substituted lower alkyl, and

n' falls in the range of 1 up to 6.

Presently most preferred compounds for use in the practice of the present invention include those wherein "Y" of Formula **I** is

$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ **(II)**,

wherein each R is selected from H, lower alkyl or substituted lower alkyl, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from H or lower alkyl; or those wherein "Y" of Formula **I** is

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ **(III)** or

$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ **(IV)**,

wherein each R is selected from H, lower alkyl or substituted lower alkyl, R' is selected from H, lower alkyl, or an hydroxy group, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from H or lower alkyl.

Referring to the structural formulae set forth above, prostaglandin-D_2 (Pg-D2) is described by Formula **I** (as set forth above), wherein A is 9-OH, Y is **IV**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4; prostaglandin-J_2 (Pg-J2) is described by Formula **I**, wherein A is absent, Y is **IV**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z'

is methyl, n is 1 and n' is 4; Δ^{12}-prostaglandin-J_2 (Δ^{12}-Pg-J2) is described by Formula I, wherein A is absent, Y is III, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4; 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-deoxy-$\Delta^{12,14}$-Pg-J2) is described by Formula I, wherein A is absent, Y is II, each R is hydrogen, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4.

The above-described compounds can be readily prepared using a variety of synthetic methods, as are well known by those of skill in the art. For example, many of the above-described compounds can be prepared chemically or enzymatically, from the naturally occurring precursor, arachidonic acid.

As employed herein, the term "modulate" refers to the ability of a modulator for a member of the steroid/thyroid superfamily to either directly (by binding to the receptor as a ligand) or indirectly (as a precursor for a ligand or an inducer which promotes production of ligand from a precursor) induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes mediated by PPARγ" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptor or receptor combinations which are responsive to the PPAR-γ-selective prostaglandin or prostaglandin-like compounds described herein. Such processes include cell differentiation to produce lipid-accumulating cells, modulation of blood glucose levels and insulin sensitivity, regulation of leptin levels and subsequent feeding levels (for the control of satiety and/or appetite), regulation of thermogenesis and fatty

acid metabolism, regulation of fat levels for the treatment of lipodystrophies, control of cell differentiation for the treatment of myxoid liposarcomas, regulation of triglyceride levels and lipoproteins for the treatment of hyperlipidemia, modulation of genes expressed in adipose cells (e.g., leptin, lipoprotein, lipase, uncoupling protein, and the like), and the like.

In accordance with the present invention, modulation of processes mediated by PPARγ can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

PPAR-γ-selective prostaglandin or prostaglandin-like compounds contemplated for use in the practice of the present invention can be employed for both *in vitro* and *in vivo* applications. For *in vivo* applications, the invention compounds can be incorporated into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when compounds contemplated for use in the practice of the present invention are so used.

In accordance with another embodiment of the present invention, there is provided a method of testing compound(s) for the ability to regulate the transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

Hormone response elements contemplated for use in the practice of the present invention are composed of at least one direct repeat of two or more half sites separated by a spacer of one nucleotide. The spacer nucleotide can be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Presently preferred response elements contain at least one copy (with one, two or three copies most common) of the minimal sequence:

AGGACA A AGGTCA (SEQ ID NO:4).

As noted above, the minimal sequence can optionally be flanked by additional residues, for example, as in the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ ID NO:5).

In a preferred embodiment of the present invention, only the ligand binding domain of PPARγ is utilized, in combination with the DNA binding domain of GAL4 protein, for the identification of PPARγ ligands or ligand-precursors. This allows one to avoid possible background signal caused by the potential presence of endogenous PPARγ in the host cells used for the assay.

The DNA binding domain of the yeast GAL4 protein comprises at least the first 74 amino acids thereof (see, for example, Keegan et al., Science 231:699-704 (1986)). Preferably, the first 90 or more amino acids of the GAL4 protein will be used, with the first 147 amino acid residues of yeast GAL4 being presently most preferred.

The GAL4 fragment employed in the practice of the present invention can be incorporated into any of a number of sites within the PPARγ receptor protein. For example, the GAL4 DNA binding domain can be introduced at the amino terminus of the PPARγ receptor protein, or the GAL4 DNA binding domain can be substituted for the native DNA binding domain of the PPARγ receptor, or the GAL4 DNA binding domain can be introduced at the carboxy terminus of the PPARγ receptor protein, or at other positions as can readily be determined by those of skill in the art. Thus, for example, a modified receptor protein can be prepared which consists essentially of amino acid residues 1-147 of GAL4, plus the ligand binding domain of PPARγ (i.e., containing the ligand binding domain only of said receptor (i.e., residues 163-475 of SEQ ID NO:1),

substantially absent the DNA binding domain and amino terminal domain thereof).

Identification methods according to the present invention involve the use of a functional bioassay system, wherein the modified receptor and a reporter plasmid are cultured in suitable host cells in the presence of test compound. Evidence of transcription (e.g., expression) of reporter gene is then monitored to determine the presence of an activated receptor-ligand complex. Accordingly, the functional bioassay system utilizes two plasmids: an "expression" plasmid and a "reporter" plasmid. The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the desired form of PPARγ receptor protein (i.e., intact receptor or GAL4 chimeric receptor as described hereinabove), in a suitable host cell. The reporter plasmid can be any plasmid which contains an operative PPRE or GAL4 response element, as appropriate, functionally linked to an operative reporter gene.

Exemplary PPREs have been described in detail hereinabove. Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:6),

such as, for example, 17MX, as described by Webster et al., in Cell 52:169-178 (1988), as well as derivatives thereof. Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary

promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)]. The plasmids pGMCAT, pGHCAT, pTK-GAL_p3-LUC, ΔMTV-GAL_p3-LUC, ΔMTV-GAL_p3-CAT, and the like, are examples of reporter plasmids which contain an operative hormone responsive promoter/enhancer element functionally linked to an operative reporter gene, and can therefore be used in the above-described functional bioassay (see Example 2 for details on the preparation of these plasmids). In pGMCAT, the operative hormone responsive promoter/enhancer element is the MTV LTR; in pGHCAT it is the functional portion of the growth hormone promoter. In both pGMCAT and GHCAT the operative reporter gene is the bacterial gene for chloramphenicol acetyltransferase (CAT).

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "PPRE," "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "PPRE" or "GAL4 response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence

and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the PPRE or GAL4 response element of the reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, in contrast to the requirements of prior art assay systems, when GAL4 chimerics are employed, there is no need to use receptor-negative cells in carrying out the invention process. Since the modified receptor employed in the practice of the present invention is the only species in the test cell which is capable of initiating transcription from a GAL4 response element, the expression of native receptor by the test cell does not contribute to background levels. Thus, the invention bioassay can be made to be very selective.

Cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor

produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with another embodiment of the present invention, there is provided a method of screening for antagonists of PPARγ receptor proteins, said method comprising

culturing test cells containing

(i) increasing concentrations of at least one compound whose ability to inhibit the transcription activation activity of PPARγ agonists is sought to be determined, and

(ii) optionally, at least one PPARγ agonist,

wherein said test cells contain

(i) exogenous DNA which expresses intact PPARγ or a modified form of PPARγ, wherein the modified form of PPARγ contains the DNA binding domain of GAL4, and

(ii) a PPRE or GAL4 response element, respectively, operatively linked to a reporter gene; and thereafter

assaying for evidence of transcription of said reporter gene in said cells as a function of the concentration of said compound in said culture medium, thereby indicating the ability of said compound to inhibit activation of transcription by PPARγ agonists.

Media employed for such culturing may include agonist for the receptor being tested, or the receptor may be constitutive (i.e., not require the presence of agonist for activation), or a fixed concentration of agonist can be added to the media employed for such testing.

The above-described assays of the present invention have low background and a broad dynamic range.

In accordance with yet another embodiment of the present invention, there is provided a method for preventing obesity, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist effective to block cell differentiation to produce lipid-accumulating cells.

As employed here, "obesity" refers generally to individuals who are at least about 20-30% over the average weight for his/her age, sex and height. Technically, "obese" is defined, for males, as individuals whose body mass index is greater than 27.8 kg/m^2, and for females, as individuals whose body mass index is greater than 27.3 kg/m^2.

Those of skill in the art recognize that there are numerous cell types which are capable of differentiation to produce "lipid-accumulating cells,"

such as, for example, mesenchymal cells (e.g., fibroblasts).

As employed herein, the phrase "amount... effective to block cell differentiation" refers to levels of compound sufficient to provide circulating concentrations high enough to effect activation of PPARγ. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range of about 100 nM up to 200 nM being preferred.

In accordance with a particular embodiment of the present invention, compositions comprising at least one prostaglandin or prostaglandin-like compound (as described above), and a pharmaceutically acceptable carrier are contemplated. Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable

organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

In accordance with still another embodiment of the present invention, there is provided a method for treating diabetes, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) agonist effective to lower the blood glucose level of said subject.

As employed herein, the phrase "amount... effective to lower blood glucose levels" refers to levels of compound sufficient to provide circulating concentrations high enough to accomplish the desired effect. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range of about 100 nM up to 200 nM being preferred.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Preparation of GAL4-receptor fusion proteins

A basic vector useful for the generation of GAL4-receptor fusion proteins is called pCMX-GAL4 (see SEQ ID NO:2). This vector encodes GAL4 DNA binding

domain, followed by a polylinker sequence useful in the cloning. The parental expression vector pCMX has been described by Umesono et al., in Cell 65:1255-1266 (1991), and the GAL4 portion of pCMX-GAL4 is derived from plasmid pSG424, described by Sadowski and Ptashne, in Nucleic Acids Res. 17:7539 (1989).

In general, GAL4-receptor ligand binding domain fusions are prepared by taking advantage of mutant receptor cDNA clones, such as GR-RAR chimera [see, for example, Giguere et al., in Nature 330:624-629 (1987)]. These mutant receptor cDNAs encode common *Xho*I sites at the end of the DNA binding domain, as described by Giguere et al., supra. To do so, a new pCMX-GAL4 vector was prepared which encodes a compatible *Sal*I site in the polylinker sequence (there is an *Xho*I site in the GAL4 sequence):

*Sal*I site: G'TCGAC

*Xho*I site: C'TCGAG

This allows efficient transfer of the receptor ligand binding domain to GAL4 DNA binding domain. Through this method, a number of chimeric species have been generated, including GAL4-PPARγ, containing residues 163-475 of PPARγ (see SEQ ID NO:1).

If mutants of the type referred to above are not available for the construction of GAL4-containing chimerics, one may simply look for any convenient restriction enzyme site within or downstream of the DNA binding domain of the receptor of interest (i.e., within about the first 30 amino acid residues downstream of the conserved Gly-Met residues of the DNA binding domain, i.e., within 30 residues of the last two residues shown in SEQ ID NO:1), and utilize the carboxy terminal sequences therefrom.

Example 2
Preparation of reporter constructs

Various reporter constructs are used in the examples which follow. They are prepared as follows:

TK-LUC: The MTV-LTR promoter sequence was removed from the MTV-LUC plasmid described by Hollenberg and Evans in Cell 55:899-906 (1988) by *Hin*dIII and *Xho*I digest, and cloned with the *Hin*dIII-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in Nucleic Acids Res. 15:5490 (1987)) to generate parental construct TK-LUC.

pTK-PPRE3-LUC: Three copies of double-stranded peroxisome proliferator response element (PPRE) oligonucleotides (see SEQ ID NO:3) were cloned upstream of the TK promoter of TK-LUC at the *Sal*I site.

pTK-MH100x4-LUC: Four copies of double-stranded MH100 oligonucleotides, encoding a GAL4 binding site, were cloned upstream of the TK promoter of TK-LUC at the *Hin*dIII site.

CMX-βGAL: The coding sequence for the *E. coli* β-galactosidase gene was isolated from plasmid pCH110 [see Hall et al., J. Mol. Appl. Genet. 2:101-109 (1983)] by *Hin*dIII and *Bam*HI digest, and cloned into pCMX eucaryotic expression vector [see Umesono et al., supra].

Example 3
Screening assay for receptor selective agonists

CV-1 cells are co-transfected with CMX-GAL-PPARγ and pTK-MH100x4-LUC at a ratio of about 100 ng of

receptor-encoding DNA per 10^5 cells. The usual amounts of DNA per 10^5 cells are 100 ng of CMX-GAL-PPARγ, 300 ng of pTK-MH100x4-LUC, and 500 ng of CMX-βGAL. Typically, transfections are performed in triplicate. The plates are then incubated for 2-3 hours at 37°C.

The cells are washed with fresh medium. Fresh medium containing one concentration of a serial dilution of agonist is added to each well. A typical agonist dilution series extends from 10^{-5}M through 10^{-11}M. A solvent control is performed for each agonist. The cells are incubated at 37°C for 1-2 days.

The cells are rinsed twice with buffered saline solution. Subsequently, cells are lysed, *in situ*, by adding 200 µl of lysis buffer. After 30 minutes incubation at room temperature, 40 µl aliquots of cell lysate are transferred to 96-well plates for luciferase reporter gene assays and β-galactosidase transfection controls [see Heyman et al., Cell 68:397-406 (1992)].

The data are expressed as relative light units (RLUs) per O.D. unit of β-galactosidase per minute. The triplicates are averaged for each concentration and plotted (see Figure 1) as fold induction induced by a standard dose (10µM) of agonist.

Example 4

Dose response of GAL4-PPARγ constructs to various prostaglandins

Effector plasmid, reporter plasmid, and β-galactosidase control plasmid are co-transfected into CV-1 cells at a ratio of about 1:3:5, using a liposome-mediated method, employing N-{2-(2,3)-dioleoyloxy)propyl-N,N,N-trimethyl ammonium methyl sulfate} (i.e., DOTAP, Boehringer Mannheim) according to the manufacturer's

instructions in Dulbecco's modified Eagle's medium (DMEM) with 10% delipidated hormone-depleted fetal calf serum. After about 2-3 hours, the cells are washed with DMEM and an appropriate prostaglandin is added to the media to the final molar concentration indicated in Figure 2. After 24-48 hours of incubation, the cells are rinsed with phosphate buffered saline (pH 7.2) and lysed. Aliquots are assayed for luciferase and β-galactosidase activity. Luciferase activity is normalized to optical density units of β-galactosidase per minute of incubation.

The data are expressed in Figure 2 as fold induction over the same construct incubated in solvent alone. Review of Figure 2 reveals that PGD2 and PGJ2 families of compounds are functional modulators of PPARγ.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Evans, Ronald M.
Forman, Barry M.

(ii) TITLE OF INVENTION: SELECTIVE MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/465,375
(B) FILING DATE: 05-JUN-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/428,559
(B) FILING DATE: 25-APR-1995

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(A) NAME: Reiter, Stephen E.
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(C) REFERENCE/DOCKET NUMBER: P41 90001

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-546-1995
(B) TELEFAX: 619-546-9392

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2005 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 352..1776

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCGAATCCC GCGCCCCAGG CGCTGCCGCT CTGAGTGCGA CGGGCCCCGC CTGGCCGGCC 60

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GGAGGACGCG GAAGAAGAGA CCTGGGGCGC TGCCTGGGGT ATTGGGTCGC GCGCAGTGAG      120

GGGACCGAGT GTGACGACAA GGTGACCGGG CTGAGGGGAC GGGCTGAGGA GAAGTCACAC      180

TCTGACAGGA GCCTGTGAGA CCAACAGCCT GACGGGGTCT CGGTTGAGGG GACGCGGGCT      240

GAGAAGTCAC GTTCTGACAG GACTGTGTGA CAGACAAGAT TTGAAAGAAG CGGTGAACCA      300

CTGATATTCA GGACATTTTT AAAAACAAGA CTACCCTTTA CTGAAATTAC C ATG GTT       357
                                                          Met Val
                                                            1

GAC ACA GAG ATG CCA TTC TGG CCC ACC AAC TTC GGA ATC AGC TCT GTG         405
Asp Thr Glu Met Pro Phe Trp Pro Thr Asn Phe Gly Ile Ser Ser Val
  5                  10                  15

GAC CTC TCC GTG ATG GAA GAC CAC TCG CAT TCC TTT GAC ATC AAG CCC         453
Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe Asp Ile Lys Pro
       20                  25                  30

TTT ACC ACA GTT GAT TTC TCC AGC ATT TCT GCT CCA CAC TAT GAA GAC         501
Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Ala Pro His Tyr Glu Asp
  35                  40                  45                  50

ATT CCA TTC ACA AGA GCT GAC CCA ATG GTT GCT GAT TAC AAA TAT GAC         549
Ile Pro Phe Thr Arg Ala Asp Pro Met Val Ala Asp Tyr Lys Tyr Asp
                55                  60                  65

CTG AAG CTC CAA GAA TAC CAA AGT GCG ATC AAA GTA GAA CCT GCA TCT         597
Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val Glu Pro Ala Ser
            70                  75                  80

CCA CCT TAT TAT TCT GAA AAG ACC CAG CTC TAC AAC AGG CCT CAT GAA         645
Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn Arg Pro His Glu
  85                  90                  95

GAA CCT TCT AAC TCC CTC ATG GCC ATT GAG TGC CGA GTC TGT GGG GAT         693
Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys Gly Asp
       100                 105                 110

AAA GCA TCA GGC TTC CAC TAT GGA GTT CAT GCT TGT GAA GGA TGC AAG         741
Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys
115                 120                 125                 130

GGT TTT TTC CGA AGA ACC ATC CGA TTG AAG CTT ATT TAT GAT AGG TGT         789
Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp Arg Cys
                135                 140                 145

GAT CTT AAC TGC CGG ATC CAC AAA AAA AGT AGA AAT AAA TGT CAG TAC         837
Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys Gln Tyr
            150                 155                 160

TGT CGG TTT CAG AAG TGC CTT GCT GTG GGG ATG TCT CAC AAT GCC ATC         885
Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser His Asn Ala Ile
  165                 170                 175

AGG TTT GGG CGG ATG CCA CAG GCC GAG AAG GAG AAG CTG TTG GCG GAG         933
Arg Phe Gly Arg Met Pro Gln Ala Glu Lys Glu Lys Leu Leu Ala Glu
       180                 185                 190

ATC TCC AGT GAT ATC GAC CAG CTG AAC CCA GAG TCT GCT GAT CTG CGA         981
Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp Leu Arg
195                 200                 205                 210

GCC CTG GCA AAG CAT TTG TAT GAC TCA TAC ATA AAG TCC TTC CCG CTG        1029
Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu
                215                 220                 225
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ACC AAA GCC AAG GCG AGG GCG ATC TTG ACA GGA AAG ACA ACG GAC AAA      1077
Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys
            230                 235                 240

TCA CCA TTT GTC ATC TAC GAC ATG AAT TCC TTA ATG ATG GGA GAA GAT      1125
Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp
        245                 250                 255

AAA ATC AAG TTC AAA CAT ATC ACC CCC CTG CAG GAG CAG AGC AAA GAG      1173
Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu Gln Ser Lys Glu
    260                 265                 270

GTG GCC ATC CGA ATT TTT CAA GGG TGC CAG TTT CGA TCC GTA GAA GCC      1221
Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala
275                 280                 285                 290

GTG CAA GAG ATC ACA GAG TAT GCC AAA AAT ATC CCT GGT TTC ATT AAC      1269
Val Gln Glu Ile Thr Glu Tyr Ala Lys Asn Ile Pro Gly Phe Ile Asn
            295                 300                 305

CTT GAT TTG AAT GAC CAA GTG ACT CTG CTC AAG TAT GGT GTC CAT GAG      1317
Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu
        310                 315                 320

ATC ATC TAC ACG ATG CTG GCC TCC CTG ATG AAT AAA GAT GGA GTC CTC      1365
Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gly Val Leu
    325                 330                 335

ATC TCA GAG GGC CAA GGA TTC ATG ACC AGG GAG TTC CTC AAA AGC CTG      1413
Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu
340                 345                 350

CGG AAG CCC TTT GGT GAC TTT ATG GAG CCT AAG TTT GAG TTT GCT GTG      1461
Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val
355                 360                 365                 370

AAG TTC AAT GCA CTG GAA TTA GAT GAC AGT GAC TTG GCT ATA TTT ATA      1509
Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile
            375                 380                 385

GCT GTC ATT ATT CTC AGT GGA GAC CGC CCA GGC TTG CTG AAC GTG AAG      1557
Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys
        390                 395                 400

CCC ATC GAG GAC ATC CAA GAC AAC CTG CTG CAG GCC CTG GAA CTG CAG      1605
Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu Glu Leu Gln
    405                 410                 415

CTC AAG CTG AAT CAC CCA GAG TCC TCT CAG CTG TTC GCC AAG GTG CTC      1653
Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe Ala Lys Val Leu
420                 425                 430

CAG AAG ATG ACA GAC CTC AGG CAG ATC GTC ACA GAG CAC GTG CAG CTA      1701
Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu His Val Gln Leu
435                 440                 445                 450

CTG CAT GTG ATC AAG AAG ACA GAG ACA GAC ATG AGC CTT CAC CCC CTG      1749
Leu His Val Ile Lys Lys Thr Glu Thr Asp Met Ser Leu His Pro Leu
            455                 460                 465

CTC CAG GAG ATC TAC AAG GAC TTG TAT TAGCAGGAAA GTCCCACCCG            1796
Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
        470                 475

CTGACAACGT GTTCCTTCTA TTGATTGCAC TATTATTTTG AGGGAAAAAA ATCTGACACC    1856
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TAAGAAATTT ACTGTGAAAA AGCATTTAAA AACAAAAAGT TTTAGAACAT GATCTATTTT 1916

ATGCATATTG TTTATAAAGA TACATTTACA ATTTACTTTT AATATTAAAA ATTACCACAT 1976

TATAAAAAAA AAAAAAAAAA AGGAATTCC 2005

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGGAGACCCA AGCTTGAAGC AAGCCTCCTG AAAG ATG AAG CTA CTG TCT TCT 52
Met Lys Leu Leu Ser Ser
1 5

ATC GAA CAA GCA TGC GAT ATT TGC CGA CTT AAA AAG CTC AAG TGC TCC 100
Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu Lys Lys Leu Lys Cys Ser
10 15 20

AAA GAA AAA CCG AAG TGC GCC AAG TGT CTG AAG AAC AAC TGG GAG TGT 148
Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu Lys Asn Asn Trp Glu Cys
25 30 35

CGC TAC TCT CCC AAA ACC AAA AGG TCT CCG CTG ACT AGG GCA CAT CTG 196
Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro Leu Thr Arg Ala His Leu
40 45 50

ACA GAA GTG GAA TCA AGG CTA GAA AGA CTG GAA CAG CTA TTT CTA CTG 244
Thr Glu Val Glu Ser Arg Leu Glu Arg Leu Glu Gln Leu Phe Leu Leu
55 60 65 70

ATT TTT CCT CGA GAA GAC CTT GAC ATG ATT TTG AAA ATG GAT TCT TTA 292
Ile Phe Pro Arg Glu Asp Leu Asp Met Ile Leu Lys Met Asp Ser Leu
75 80 85

CAG GAT ATA AAA GCA TTG TTA ACA GGA TTA TTT GTA CAA GAT AAT GTG 340
Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu Phe Val Gln Asp Asn Val
90 95 100

AAT AAA GAT GCC GTC ACA GAT AGA TTG GCT TCA GTG GAG ACT GAT ATG 388
Asn Lys Asp Ala Val Thr Asp Arg Leu Ala Ser Val Glu Thr Asp Met
105 110 115

CCT CTA ACA TTG AGA CAG CAT AGA ATA AGT GCG ACA TCA TCA TCG GAA 436
Pro Leu Thr Leu Arg Gln His Arg Ile Ser Ala Thr Ser Ser Ser Glu
120 125 130

GAG AGT AGT AAC AAA GGT CAA AGA CAG TTG ACT GTA TCG CCG GAA TTC 484
Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu Thr Val Ser Pro Glu Phe
135 140 145 150

CCG GGG ATC CGT CGA CGG TAC CAG ATA TCA GGA TCC TGG CCA GCT AGC 532
Pro Gly Ile Arg Arg Arg Tyr Gln Ile Ser Gly Ser Trp Pro Ala Ser
155 160 165

TAG GTA GCT AGA GG 546
* Val Ala Arg
170

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
130 135 140

Thr Val Ser Pro Glu Phe Pro Gly Ile Arg Arg Arg Tyr Gln Ile Ser
145 150 155 160

Gly Ser Trp Pro Ala Ser * Val Ala Arg
165 170

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGGACAAAGG TCA

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGACCAGGAC AAAGGTCACG TTC 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGAGGACTG TCCTCCG 17

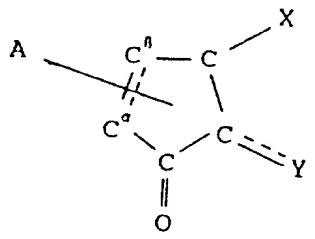
That which is claimed is:

1. A method for modulating process(es) mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising conducting said process(es) in the presence of at least one PPAR-γ-selective prostaglandin or prostaglandin-like compound or precursor thereof.

2. A method according to Claim 1 wherein said PPAR-γ-selective prostaglandin is selected from a prostaglandin-J_2, a prostaglandin-D_2, or a precursor thereof.

3. A method according to Claim 2 wherein said prostaglandin-J_2 is selected from prostaglandin-J_2, Δ^{12}-prostaglandin-J_2 or 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2.

4. A method according to Claim 1, wherein said PPAR-γ-selective prostaglandin or prostaglandin-like compound has the structure I:



(I)

wherein:

A is selected from hydrogen or a leaving group at the α- or β- position of the ring, or A is absent when there is a double bond between C^{α} and C^{β} of the ring;

X is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or

substituted alkynyl group having in the range of 2 up to 15 carbon atoms; and

Y is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl group having in the range of 2 up to 15 carbon atoms.

5. A method according to claim 4 wherein:

X of Formula I is selected from:

$-(CRR)_m-Z$,

$-(CRR)_{m'}-C(R)=C(R)-(CRR)_{m'}-Z$, or

$-(CRR)_{m''}-C\equiv C-(CRR)_{m''}-Z$, wherein:

each R is independently selected from hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

m falls in the range of 1 up to 15,

each m' falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkenyl moiety does not exceed 15 carbon atoms,

each m" falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkynyl moiety does not exceed 15 carbon atoms, and

Z is a polar, heteroatom-containing substituent; and

Y of Formula I is selected from:

$=C(R)-[C(R)=C(R)]_n-(CRR)_{n'}-Z'$ (II),

$=C(R)-[C\equiv C]_{n''}-(CRR)_{n'}-Z'$ (IIA),

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ (III),

$-[C(R)=C(R)]_n-(CRR)_{n'}-Z'$ (IV),

$-[C\equiv C]_n-(CRR)_{n'}-Z'$ (IVA),

wherein

each R is independently as defined above,

each R' is independently selected from H, lower alkyl, substituted lower alkyl, or a leaving group,

Z' is selected from H, lower alkyl or substituted lower alkyl,

n falls in the range of 0 up to 4,

n' falls in the range of 2 up to 12, and

n" falls in the range of 1 up to 3.

6. A method according to claim 5 wherein Z is selected from cyano, nitro, amino, carbamate, or a substituent having the structure:

$-CH_2OR'$, wherein R' is selected from H, alkyl, alkenyl, alkynyl, acyl or aryl;

-C(O)R", wherein R" is selected from H, alkyl, substituted alkyl, alkoxy, alkylamino, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aryloxy, arylamino, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, heterocyclic, substituted heterocyclic or trifluoromethyl,

$-CO_2R'''$, wherein R''' is selected from H, alkyl, alkenyl or alkynyl;

$-SR'$, $-S(O)R'$, $-S(O)_2R'$ or $-S(O)_2NHR'$, wherein each R' is as defined above.

7. A method according to claim 5 wherein:

X of Formula I is $-CRR-C(R)=C(R)-(CRR)_m-Z$, wherein:
each R is independently selected from hydrogen, lower alkyl, substituted lower alkyl, hydroxy, alkoxy (of a lower alkyl group), halogen, trifluoromethyl, amino, carboxyl, or sulfonyl,
m falls in the range of 1 up to 6, and
Z is selected from $-CH_2OH$, $-CH_2OAc$, $-CO_2H$, $-CO_2Me$ or $-CO_2Et$; and

Y of Formula I is selected from:
$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ (II),
$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ (III), or
$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ (IV), wherein
each R is independently as defined above,
each R' is independently selected from H, lower alkyl, substituted lower alkyl, or a leaving group,
Z' is selected from H, lower alkyl or substituted lower alkyl, and
n' falls in the range of 1 up to 6.

8. A method according to claim 7 wherein Y of Formula I is

$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ (II),

wherein each R is selected from hydrogen, lower alkyl or substituted lower alkyl, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from hydrogen or lower alkyl.

9. A method according to claim 7 wherein Y of Formula **I** is

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ **(III)** or

$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ **(IV)**,

wherein each R is selected from hydrogen, lower alkyl or substituted lower alkyl, R' is selected from hydrogen, lower alkyl, or an hydroxy group, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from hydrogen or lower alkyl.

10. A method according to claim 5 wherein A is 9-OH, Y is **IV**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m = 3, Z' is methyl, n = 1 and n' = 4.

11. A method according to claim 5 wherein A is absent, Y is **IV**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z' is methyl, n = 1 and n' = 4.

12. A method according to claim 5 wherein A is absent, Y is **II**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m = 3, Z' is methyl, n = 1 and n' = 4.

13. A method according to claim 5 wherein A is absent, Y is **I**, each R is hydrogen, Z is $-CO_2H$, m = 3, Z' is methyl, n = 1 and n' = 4.

14. A method according to claim 1 wherein said process mediated by PPAR-γ is cell differentiation to produce lipid-accumulating cells.

15. A method according to claim 1 wherein said process mediated by PPAR-γ is the response of the recipient to insulin.

16. A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

17. A method according to Claim 16 wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

18. A method according to claim 17 wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA,

wherein said minimal sequence is optionally flanked by additional residues.

19. A method according to claim 17 wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC.

20. A method according to claim 16 wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma.

21. A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ-selective modulator.

22. A method for preventing obesity, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist effective to block cell differentiation to produce lipid-accumulating cells.

23. A method for treating diabetes, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) agonist effective to lower the blood glucose level of said subject.

09/155252

FIGURE 1

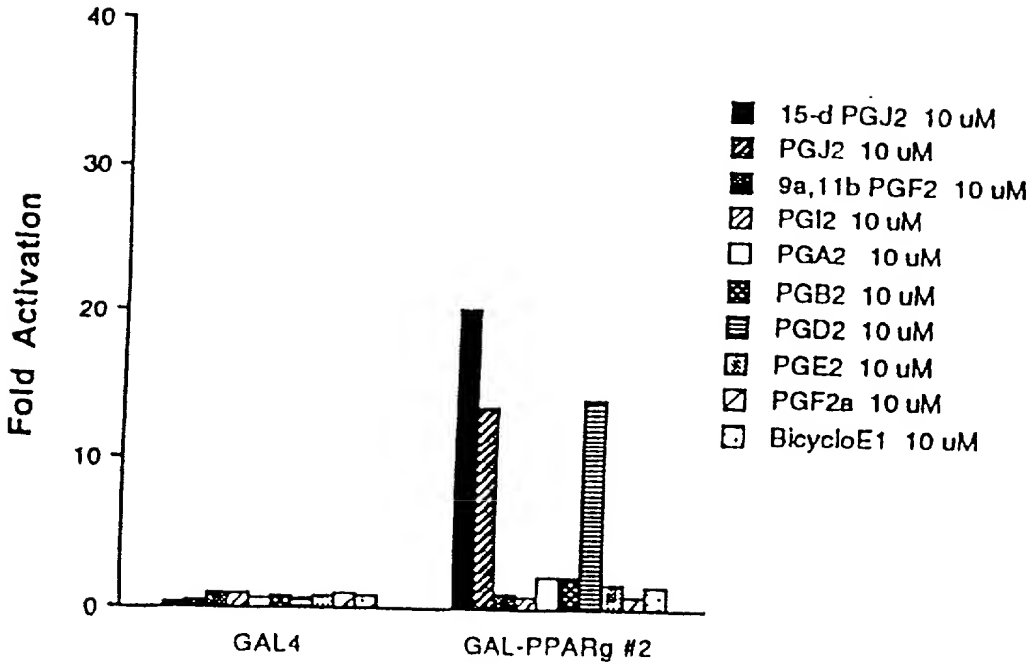
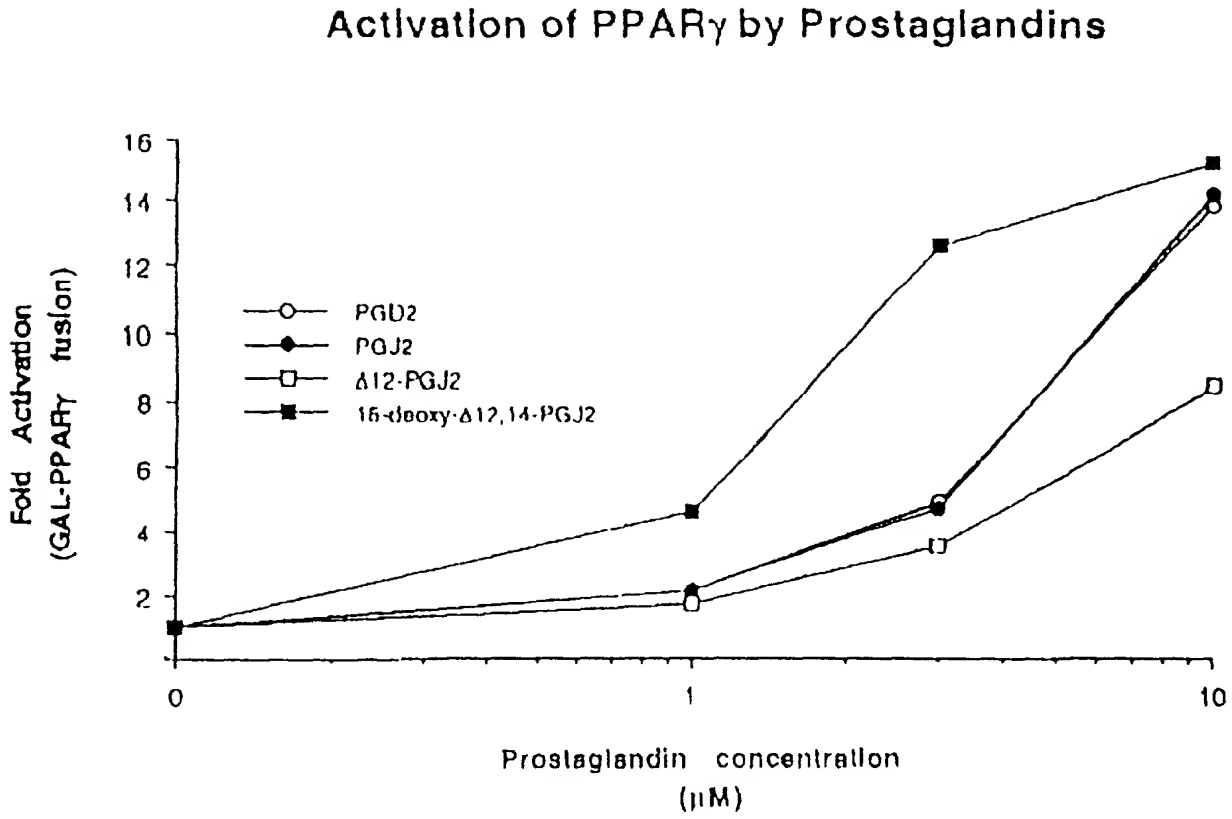


FIGURE 2


Activation of PPARγ by Prostaglandins
Fold Activation (GAL-PPARγ fusion)
16
14
12
10
8
6
4
2
0
1
10
PGD2
PGJ2
Δ12-PGJ2
16-deoxy-Δ12,14-PGJ2
Prostaglandin concentration (μM)

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As the below-named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names.

We believe we are an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled **SELECTIVE MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF**, the specification of which

__ is attached hereto.

_x_ was filed on April 18, 1996 (Attorney Docket No.SALK1470-2) as PCT Application Serial No. PCT\US96\05465 and was amended on (or amended through) _____.
(if applicable)

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
P419926 08/428,559	4/25/95	Pending
P4190001 08/465,375	6/5/95	Pending
PCT\US96\05465	4/18/96	Completed

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

STEPHEN E. REITER, Registration No. 31,192; GREGORY P. RAYMER, Registration No. 36,647; DAVID F. KLEINSMITH, Registration No. 40,050; BARRY N. YOUNG, Registration No. 27,774; TIMOTHY W. LOHSE, Registration No. 35,255; STANLEY H. KIM, Registration No. 40,047; RAMSEY R. STEWART, Registration No. 38,322, JUNE LEARN, Registration No. 31, 238, ROBROY R. FAWCETT, Registration No. 35,133, DARLENE HAYES, Registration No. 33,899, WILLIAM N. HULSEY III, Registration No. 33,402; STEVEN R. SPRINKLE, Registration No. 40,825; and TERRANCE A. MEADOR, Registration No. 30,298.

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Inventor's signature: _____

Date: _____

Residence: 1471 Cottontail Lane
La Jolla, Ca. 92037

Citizenship: U.S.

Post Office Address:

Full name of second inventor: Barry Marc Forman

Inventor's signature: Barry Marc Forman

Date: 5/27/98

Residence: 1671 S. Diamond Bar Blvd.
Diamond Bar, Ca. 91765

Citizenship: U.S.

Post Office Address:

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Inventor's signature: Ronald M Evans

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Citizenship: U.S.

Post Office Address:

Full name of second inventor: Barry Marc Forman

Inventor's signature:_____

Date: _____

Residence: 1671 S. Diamond Bar Blvd.
Diamond Bar, Ca. 91765

Citizenship: U.S.

Post Office Address:

Attorney Docket No.: **SALK 1470-2**
Applicant or Patentee: **Evans et al.**
Serial No. or Patent No.: **Unassigned**
Filed : **Herewith**

Title: **SELECTIVE MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF**

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. §§1.9(f) and 1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: **THE SALK INSTITUTE FOR BIOLOGICAL STUDIES**
ADDRESS OF ORGANIZATION: **10010 NORTH TORREY PINES ROAD, LA JOLLA, CALIFORNIA 92037**

TYPE OF ORGANIZATION

- ☐ University or other Institution of Higher Education
- ☒ Tax Exempt under Internal Revenue Service Code (26 U.S.C. §§501(a) and 501(c) (3))
- ☐ Nonprofit Scientific or Educational under Statute of State of the United States of America (Name of State _____) (Citation of Statute _____)
- ☐ Would qualify as tax exempt under Internal Revenue Service Code (26 U.S.C. §§501(a) and 501(c) (3)) if located in the United States of America
- ☐ Would qualify as nonprofit Scientific or Educational under Statute of State of the United States of America if located in the United States of America (Name of State _____) (Citation of Statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. §1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, with regard to the invention entitled **SELECTIVE MODULATORS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AND METHODS FOR THE USE THEREOF** by inventor(s) **Ronald M. Evans and Barry M. Forman** described in:

- ☒ the specification filed herewith
- ☒ Based on PCT\Application Serial No. PCT\US96\05465, filed April 18, 1996.
- ☐ Patent No. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. §1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. §1.9(d) or a nonprofit organization under 37 C.F.R. §1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 C.F.R. §1.27).

Full Name _____
Address _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Full Name _____
Address _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Full Name _____
Address _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. §1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: T. D. Ballard
TITLE IN ORGANIZATION: President
ADDRESS OF PERSON SIGNING: **10010 North Torrey Pines Road, La Jolla, CA 92037**
SIGNATURE: T D Ballard Date: 5/25/98

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62574-995470